

## «TrickleZyme» Project

# PFI Consolidates Its Enzyme Production Expertise

*«Growth Limited and Continuous Enzyme Production in a Trickle Bed Reactor» is the name of a project jointly initiated by PFI Biotechnology and Oklahoma State University (OSU) in early June 2015. The project has a duration of three years and is funded by the German Federal Ministry of Education and Research through its Bioeconomy International program. The main focus of study is on cellulase production by enzyme-producing filamentous fungi, which cease to grow when the culture medium is modified yet continue to produce enzymes.*

The European Union is actively promoting development of a sustainable, bio-based economy with the set goal of producing not only foodstuffs but also materials and fuels from biomass. Achievement of this target will require cost-effective methods for expanding the possible uses of biomass.

In particular, materials and fuels should be produced from biomasses which do not conflict with food production. One such biomass is crop straw, which has long been an object of study at PFI.

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### Cellulase Production

Residual agricultural materials such as straw are not so readily utilisable as maize, sugar beet, or grain. Straw consists mainly of cellulose, hemicellulose, and lignin. Cellulose itself is a glucose polymer with amorphous and crystalline regions. Once the cellulose has been broken down to glucose, this compound can be readily converted into chemicals or biofuel by fermentation with microorganisms. In contrast, the step of cellulose cleavage proves difficult. This requires use of several enzymes belonging to the cellulase group, viz. cellobiohydrolase, endoglucanase, and beta-glucosidase. Their production is currently expensive and their cost is currently the limiting factor in the development of biorefineries which can process lignified substrates as starting materials. Optimising production of these three cellulases is at the heart of the TrickleZyme Project.

Certain species of mould fungi, also known as filamentous fungi, naturally produce cellulases. These fungi grow preferentially on solid surfaces where they form a dense flat mycelium (fungal tissue). On addition of certain substrates they release cellulases at the ends of their fine hyphae (these are threadlike fungal cells). Several studies have already demonstrated that filamentous fungi produce higher enzyme concentrations in such so-called solid-state fermentation than in liquid-state or submerged fermentation, in which fungi are more likely to grow in tangled form.

### Special Trickle-film Reactor

The advantage of greater enzyme productivity offered by solid-state fermentation can be combined with the processing advantages of liquid-state fermentation on use of a trickle-film reactor. The fungus establishes itself on a solid surface, while a liquid culture medium trickles from top to bottom down through the reactor. Air is blown in at the bottom to supply oxygen and moves upwards in countercurrent mode. In this way, the fungus receives an optimum supply of nutrients and oxygen and can continue its natural growth on a solid surface. This concept also allows collection of excreted enzymes in the liquid medium at the bottom of the reactor. Since the fungus is firmly attached to the solid surface the product stream contains little fungal mass which has to be separated later than it would in liquid phase fermentation.

### “Change of Diet” Inhibits Growth without Limiting Enzyme Production

A remaining problem is that the fungus grows not only in the places where it should, i.e. on the dedicated surfaces in the reactor, but also in piping or on valves. This inevitably leads to fouling and blockages. A special approach is therefore adopted in which, after an initial growth phase, the fungus is fed a culture medium lacking important substances necessary for growth. The fungus can then no longer grow but continues to produce the desired enzymes.

Studies at OSU have shown that enzyme release remains unchanged despite reduced growth. Precisely because the fungus can no longer grow, it is expected that the available nutrients can be increasingly utilised for enzyme production, thus leading to higher yields and improved profitability. PFI is taking the lead in further development of the process in the TrickleZyme Project in preparation for its large-scale industrial application.



The process holds promise for the production not only of numerous other enzymes in addition to cellulases but also of other products such as proteins, antibiotics, or organic acids.

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GEFÖRDERT VOM



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The project is based on the following publications:

Müller M., R.A. Prade, F. Segato, H.K. Atiyeh, M.R. Wilkins (2015): Continuous xylanase production with *Aspergillus nidulans* under pyridoxine limitation using a trickle bed reactor. *Bioresource Technology* 188:219-225

Müller M., F. Segato, R.A. Prade, M.R. Wilkins (2014): High-yield recombinant xylanase production by *Aspergillus nidulans* under pyridoxine limitation. *Journal of Industrial Microbiology & Biotechnology* 41:1563-1570